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(54) Human Fc-gamma receptor III

(57) Soluble and membrane-bound human FcγRIII and variants are provided, together with nucleic acids encoding the same. Also described are pharmaceutical compositions containing FcγRIII or variants and the use of these proteins for treatment of immune thrombocytopenic purpura.

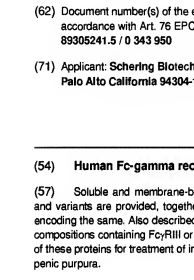

FIGURE 1 PART A

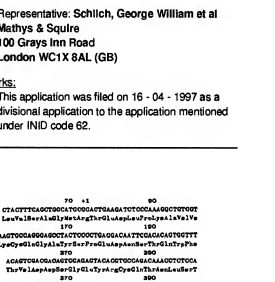

FIGURE 1 PART B

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Description

Field of the Invention

The invention relates generally to therapeutic compounds, and more particularly to soluble and membrane-bound forms of a low-affinity receptor for human immunoglobulin G, nucleic acids encoding the same, and diagnostic and therapeutic uses of such receptors.

BACKGROUND

Receptors for the Fc portion of immunoglobulin G (IgG) play a central role in cellular immune defenses. Three types of such receptors have been identified: A 72 kilodalton (kD) receptor with high affinity for monomeric IgG is found on monocytes and some macrophages, a 40 kD receptor with low affinity for monomeric IgG is found on monocytes, neutrophils, eosinophils, platelets and certain human tumor-cell lines, and a 50-70 kD receptor with low affinity for monomeric IgG is found on neutrophils, eosinophils, natural killer cells, and macrophages. These three types of $Fc\gamma$ receptor are referred to as $Fc\gamma RI$, $Fc\gamma RII$, and $Fc\gamma RIII$, respectively: Unkeless et al., Ann. Rev. Immunol., Vol. 6, pgs. 251-281 (1988).

It is believed that $Fc\gamma RIII$-mediated removal IgG-coated platelets plays an important part in the pathogenesis of immune thrombocytopenic purpura (ITP), a platelet-deficiency condition characterized by excessive bleeding: von dem Borne, pgs. 222-256, in Immunohaematology, Engelfriet et al., eds. (Elsevier, Amsterdam, 1984). Clarkson et al., New England J. Med., Vol. 314, pgs. 1236-1239 (1986), report that the infusion of ligand-blocking anti-$Fc\gamma RIII$ antibody into a patient with refractory ITP resulted in a transient increase in platelet count. This observation suggests that the most deleterious manifestation of ITP could be temporarily ameliorated by the administration of agents that block or compete with $Fc\gamma RIII$ for binding sites on IgG-coated platelets.

In a separate area of clinical immunology, elevated serum levels of aggregates consisting of immunoglobulin and antigen (so-called "immune complexes") have been correlated with a wide variety of disorders, particularly autoimmune diseases, such as systemic lupus erythematosus (SLE), and rheumatoid arthritis. The level of such complexes has become an important diagnostic for presence of autoimmune disorders; e.g. Theofilopoulos et al., Am.J. Pathol., Col. 100, pgs. 531-591 (1980).

Present assays for the serum level of immune complexes include solid-phase assays which take advantage of the affinity of the complexes for certain complement or rheumatoid factor proteins, and cellular assays which take advantage of the property of Raji cells to preferentially bind immune complexes; Theofilopoulos et al., chapter 28, and Toth et al., chapter 29, in Rose et al., eds. Manual of Clinical Immunology, 3rd Ed. (American Society for Microbiology, Washington, D.C., 1986). Unfortunately, like many mammalian-cell based assays, the Raji-cell assay is difficult to perform, and requires elaborate controls and standards because of the inherent variability of Raji-cell binding to immune complexes.

In light of the foregoing, it would be advantageous for the medical community to have alternative assay methods for immune complexes utilizing well characterized, widely available, and conveniently cultured cell lines. It would also be advantageous if soluble $Fc\gamma Rs$ could be produced in sufficient quantity to permit practical emergency therapy for refactory cases of ITP.

SUMMARY OF THE INVENTION

The present invention is directed to soluble and membrane-bound human $Fc\gamma RIII$ polypeptides, their muteins, nucleic acids capable of encoding the same, and diagnostic and therapeutic uses of such polypeptides and muteins.

The invention is based on the discovery of a cDNA encoding a human $Fc\gamma RIII$. A cDNA clone, pcD(SRα) containing the $Fc\gamma RIII$-encoding insert (illustrated in Figure 1) is deposited in E. coli K12 strain MC1061 with the American Type Culture Collection (ATCC), Rockville, Maryland, USA, under accession number 67707.

Preferred embodiments of the invention include proteins having amino acid sequences selected from the group of 2-fold substituted and 1-fold deleted sequences defined by the formulas:

X(Arg) - X(Thr) - X(Glu) - X(Asp) - X(Leu) - X(Pro) - X(Lys) - X(Ala) - X(Val) - X(Val) - X(Phe) - X(Leu) - X(Glu) - X(Pro) - X(Gln) - X(Trp) - X(Tyr) - X(Arg) - X(Val) - X(Leu) - X(Glu) - X(Lys) - X(Asp) - X(Ser) - X(Val) - X(Thr) - X(Leu) - X(Lys) - X(Cys) - X(Gln) - X(Gly) - X(Ala) - X(Tyr) - X(Ser) - X(Pro) - X(Glu) - X(Asp) - X(Asn) - X(Ser) - X(Thr) - X(Gln) - X(Trp) - X(Phe) - X(His) - X(Asn) - X(Glu) - X(Asn) - X(Leu) - X(Ile) - X(Ser) - X(Ser) - X(Gln) - X(Ala) - X(Ser) - X(Ser) - X(Tyr) - X(Phe) - X(Ile) - X(Asp) - X(Ala) - X(Ala) - X(Thr) - X(Val) - X(Asp) - X(Asp) - X(Ser) - X(Gly) - X(Glu) - X(Tyr) - X(Arg) - X(Cys) - X(Gln) - X(Thr) - X(Asn) - X(Leu) - X(Ser) - X(Thr) - X(Leu) - X(Ser) - X(Asp) - X(Pro) - X(Val) - X(Gln) - X(Leu) - X(Glu) - X(Val) - X(His) - X(Val) - X(Gly) - X(Trp) - X(Leu) - X(Leu) - X(Leu) - X(Gln) - X(Ala) - X(Pro) - X(Arg) - X(Trp) - X(Val) - X(Phe) - X(Lys) - X(Glu) - X(Glu) - X(Asp) - X(Pro) - X(Ile) - X(His) - X(Leu) - X(Arg) - X(Cys) - X(His) - X(Ser) - X(Trp) - X(Lys) - X(Asn) - X(Thr) - X(Ala) - X(Leu) - X(His) - X(Lys) - X(Val) - X(Thr) - X(Tyr) - X(Leu) - X(Gln) - X(Asn) - X(Gly) - X(Lys) - X(Asp) - X(Arg) - X(Lys) - X(Tyr) - X(Phe) - X(His) - X(His) - X(Asn) - X(Ser) - X(Asp) - X(Phe) - X(His) - X(Ile) - X(Pro) - X(Lys) - X(Ala) - X(Thr) - X(Leu) - X(Lys) - X(Asp) - X(Ser) - X(Gly) - X(Ser) - X(Tyr) - X(Phe) - X(Cys) - X(Arg) - X(Gly) - X(Leu) - X(Val) - X(Gly) - X(Ser) - X(Lys) - X(Asn) - X(Val) - X(Ser) - X(Ser) - X(Glu) - X(Thr) - X(Val) - X(Asn) - X(Ile) - X(Thr)

- X(Ile) - X(Thr) - X(Gln) - X(Gly) - X(Leu) - X(Ala) - X(Val) - X(Ser) - X(Thr) - X(Ile) - X(Ser) - X(Ser) - X(Phe) - X(Ser) - X(Pro) - X(Pro) - X(Gly)

Formula 1

and

X(Arg) - X(Thr) - X(Glu) - X(Asp) - X(Leu) - X(Pro) - X(Lys) - X(Ala) - X(Val) - X(Val) - X(Phe) - X(Leu) - X(Glu) - X(Pro) - X(Gln) - X(Trp) - X(Tyr) - X(Arg) - X(Val) - X(Leu) - X(Glu) - X(Lys) - X(Asp) - X(Ser) - X(Val) - X(Thr) - X(Leu) - X(Lys) - X(Cys) - X(Gln) - X(Gly) - X(Ala) - X(Tyr) - X(Ser) - X(Pro) - X(Glu) - X(Asp) - X(Asn) - X(Ser) - X(Thr) - X(Gln) - X(Trp) - X(Phe) - X(His) - X(Asn) - X(Glu) - X(Ser) - X(Leu) - X(Ile) - X(Ser) - X(Ser) - X(Gln) - X(Ala) - X(Ser) - X(Ser) - X(Tyr) - X(Phe) - X(Ile) - X(Asp) - X(Ala) - X(Ala) - X(Thr) - X(Val) - X(Asp) - X(Asp) - X(Ser) - X(Gly) - X(Glu) - X(Tyr) - X(Arg) - X(Cys) - X(Gln) - X(Thr) - X(Asn) - X(Leu) - X(Ser) - X(Thr) - X(Leu) - X(Ser) - X(Asp) - X(Pro) - X(Val) - X(Gln) - X(Leu) - X(Glu) - X(Val) - X(His) - X(Ile) - X(Gly) - X(Trp) - X(Leu) - X(Leu) - X(Leu) - X(Gln) - X(Ala) - X(Pro) - X(Arg) - X(Trp) - X(Val) - X(Phe) - X(Lys) - X(Glu) - X(Glu) - X(Asp) - X(Pro) - X(Ile) - X(His) - X(Leu) - X(Arg) - X(Cys) - X(His) - X(Ser) - X(Trp) - X(Lys) - X(Asn) - X(Thr) - X(Ala) - X(Leu) - X(His) - X(Lys) - X(Val) - X(Thr) - X(Tyr) - X(Leu) - X(Gln) - X(Asn) - X(Gly) - X(Lys) - X(Gly) - X(Arg) - X(Lys) - X(Tyr) - X(Phe) - X(His) - X(His) - X(Asn) - X(Ser) - X(Asp) - X(Phe) - X(Tyr) - X(Ile) - X(Pro) - X(Lys) - X(Ala) - X(Thr) - X(Leu) - X(Lys) - X(Asp) - X(Ser) - X(Gly) - X(Ser) - X(Tyr) - X(Phe) - X(Cys) - X(Arg) - X(Gly) - X(Leu) - X(Phe) - X(Gly) - X(Ser) - X(Lys) - X(Asn) - X(Val) - X(Ser) - X(Ser) - X(Glu) - X(Thr) - X(Val) - X(Asn) - X(Ile) - X(Thr) - X(Ile) - X(Thr) - X(Gln) - X(Gly) - X(Leu) - X(Ala) - X(Val) - X(Ser) - X(Thr) - X(Ile) - X(Ser) - X(Ser) - X(Phe) - X(Phe) - X(Pro) - X(Pro) - X(Gly)

Formula II

wherein the term X(Xaa) represents the group of synonymous L-amino acids to the amino acid Xaa. Synonymous amino acids within a group have sufficiently similar physiochemical properties for substitution between members of the group to preserve the biological function of the molecule, Grantham, Science, Vol. 185, pgs. 862-864 (1974); and Dayhoff et al., Atlas of Protein Sequence and Structure 1972, Vol. 5, pgs. 89-99. It is clear that deletions of amino acids may also be made in the above-identified sequence without altering biological function, particularly if only a few amino acids are deleted and amino acids that are critical to a functional conformation are not removed or displaced, e.g. some cysteine residues: Anfisen, "Principles That Govern The Folding of Protein Chains', Science, Vol. 181, pgs. 223-230 (1973). Proteins and muteins produced by deletions are within the purview of the present invention. Whenever an amino acid residue of a protein of Formula I or II is referred to herein by number, such a number is in reference to the N-terminus of the protein.

Preferred, and more preferred, groups of synonymous amino acids for each amino are listed in Table I. The respective groups for each amino acid are indicated by slashes (/). Amino acids to the left of the slash make up the most preferred group. All the amino acids on the same line make up the more preferred group.

As used herein, "N-fold substituted" in reference to Formula I or II describes a group of amino acid sequences differing from the native amino acid sequences of Formula III or IV, respectively, by zero-N substitutions, such that the replacing amino acid is selected from the appropriate group of amino acids synonymous to the amino acid at the location of the substitution. That is, if the X(Ala) is at the location for substitution, then the Ala at that location can be replaced with Ser, Thr, or Gly.

Likewise, as used herein, the term "N-fold deleted" in reference to Formula I or II describes a group of amino acid sequences differing from the native amino acid sequences of Formula III or IV, respectively, by zero-N deletions of amino acids.

Throughout, standard abbreviations are used to designate amino acids, nucleotides, restriction endonucleases, and the like; e.g. Cohn, "Nomenclature and Symbolism of α-Amino Acids," Methods in Enzymology, Vol. 106, pgs. 3-17 (1984); Wood et al. Biochemistry: A Problems Approach, 2nd ed. (Benjamin, Menlo Park, 1981); and Roberts, "Directory of Restriction Endonucleases", Methods in Enzymology, Vol. 68, pgs. 27-40 (1979).

Table I

Preferred and More Preferred Groups of Synonymous Amino Acids	
Amino Acid	Synonymous Groups
Ala	Ala Ser / Thr Gly
Arg	Arg / Lys
Asn	Asn Asp / Ser
Asp	Asp Glu / Asn
Cys	Cys
Gln	Gln / Glu
Glu	Glu Asp / Gln
Gly	Gly Ala / Ser
His	His / Asn
Ile	Ile Val / Leu
Leu	Leu Val / Ile
Lys	Lys Arg / Asn
Met	Met / Leu
Phe	Phe
Pro	Pro / Ala
Ser	Ser Ala Thr / Gly Asn
Thr	Thr Ser Ala / Lys
Trp	Trp
Tyr	Tyr
Val	Val Ile / Ala Leu

Brief Description of The Drawings

Figure 1, parts A and B (on two sheets to be read side-by-side) illustrates the nucleotide sequence of the cDNA insert of pcD(SRα)-GP5;

Figure 2 illustrates the relative location of the stop codon inserted to produce a soluble human FcγRIII, secreted as an FcγRIII mutant;

Figure 3 illustrates the result of gel electrophoretic analysis of the soluble FcγRIII produced in Example III below; and

Figure 4 parts A and B (on two sheets to be read side-by-side) illustrates the necleotide sequence of the cDNA insert of pcD(SRα)-NL10.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes nucleic acids encoding polypeptides capable of binding to the Fc portion of human IgG. These polypeptides are derived from human FcγRIII. The invention includes soluble and membrane-bound polypeptides for human FcγRIII. These and other modified versions of the polypeptides are readily produced using standard protein engineering techniques.

Once nucleic acid sequence and/or amino acid sequence information is available for a native protein, a variety of techniques become available for producing virtually any mutation in the native sequence. Shortle, in Science, Vol. 229, pgs. 1193-1201 (1985), reviews techniques for mutating nucleic acids which are applicable to the present invention. Preferably, mutants of the protein of the present invention are produced by site-specific oligonucleotide-directed mutagenesis, e.g. Zoller and Smith, Methods in Enzymology, Vol. 100, pgs. 468-500 (1983), and Mark et al., U.S. Patent 4,518,584 entitled "Human Recombinant Interleukin-2 Muteins", which are incorporated by reference; or by so-called "cassette" mutagenesis described by Wells et al. in Gene, Vol. 34, pgs. 315-323 (1985), by Estell et al. in Science, Vol. 233, pgs. 659-663 (1986), and also essentially by Mullenbach et al. in J. Biol. Chem., Vol. 261, pgs. 719-722 (1986), and by Feretti et al. in Proc. Natl. Acad. Sci., Vol. 83, pgs. 597-603 (1986).

Polypeptides with amino acid modifications (i.e. muteins) may be desirable in a variety of circumstances. For example, undesirable side effects might be reduced by certain muteins, particularly if the side effect is associated with a different part of the polypeptide from that of the desired activity. In some expression systems, the native polypeptide may be susceptible to degradation by proteases. In such cases, selected substitutions and/or deletions of amino acids which change the susceptible sequences can significantly enhance yields, e.g. British patent application 2173-804-A where Arg at position 275 of human tissue plasminogen activator is replaced by Gly or Glu. Muteins may also increase yields in purification procedures and/or increase shelf lives of proteins by eliminating amino acids susceptible to oxidation, acylation, alkylation, or other chemical modifications. For example, methionine readily undergoes oxidation to form a sulfoxide, which in many proteins is associated with loss of biological activity: e.g. Brot and Weissbach, Arch. Biochem. Biophys., Vol. 223, pg. 271 (1983). Methioninres can often be replaced by more inert amino acids with little or no loss of biological activity, e.g. Australian patent application AU-A-52451/86. In bacterial expression systems, yields can sometimes be increased by eliminating or replacing conformationally inessential cysteine residues, e.g. Mark et al., U.S. Patent 4,518,584.

Preferably, soluble forms of the FcγRIII of the invention are produced by introducing a stop codon prior to (i.e. in the 5'- or "upstream" direction of) the coding region for the transmembrane and intracellular portions of the FcγRIII cDNA. This is conveniently done by site-specific mutagenesis. Transmembrane regions are readily identified by the presence of an amino acid segment containing from about 20-25 residues having high

average hydrophobicity, e.g. Wickner, Science, Vol. 210, pgs. 861-868 (1980), and Greene et al., Ann. Rev. Immunol., Vol. 4, pgs. 69-95 (1986).

Plasmid pcD(SRα)-GP5 is similar to the pcD shuttle vector described by Okayama and Berg, Mol. Cell. Biol., Vol. 2, pgs. 161-170 (1983), and Vol. 3, pgs. 280-289 (1983), except that the SV40 promoter has been modified to improve expression by the downstream insertion of a portion of the long terminal repeat (LTR) from a HTLV(I) retrovirus, described by Takebe et al., Mol. Cell. Biol., Vol. 8, pgs. 466-472 (1988). The plasmid is conveniently propagated in E. coli K12 strain MC1061, or like host.

The immunoglobulin G binding property of the $Fc\gamma RIIIs$ of the invention is measured by standard techniques, e.g. (1) in the case of membrane-bound $Fc\gamma RIII$: the ability of cells transfected with the $Fc\gamma RIII$ cDNA to form rosettes in the presence of IgG-coated red blood cells (RBCs) or to preferentially bind human IgG aggregated by heat treatment; or (2) in the case of soluble $Fc\gamma RIII$: the ability to preferentially remove $Fc\gamma RIII$ from solution by an immunoadsorbent column comprising human IgG, or to inhibit rosette formation between IgG-coated RBCs and cells known to have $Fc\gamma RIIIs$. The former measurements can be made with fluorescently labeled human IgG molecules, e.g. Haugland, Handbook of Fluorescent Probes (Molecular Probes, Inc., Junction City, OR, 1985). The latter measurements can be made by constructing an IgG column from isolated human IgG and a commercially available activated sepharose column, e.g. from Bio-Rad Laboratories (Richmond, CA).

Rosette assays are standard in the art, e.g. Winchester et al., chapter 31, in Rose et al., eds., Manual of Clinical Laboratory Immunology, 3rd Ed. (American Society for Microbiology, Washington, D.C., 1986).

Once the cDNA of the invention has been cloned, a wide range of expression systems (i.e. combinations of host and expression vector) can be used to produce the proteins of the invention. Possible types of host cells include, but are not limited to, bacterial, yeast, insect, mammalian, and the like. Selecting an expression system, and optimizing protein production thereby, involves the consideration and balancing of many factors, including (1) the nature of the protein to be expressed, e.g. the protein may be poisonous to some host organisms, it may be susceptible to degradation by host proteases, or it may be expressed in inactive conformations or in insoluble form in some hosts, (2) the nature of the messenger RNA (mRNA) corresponding to the protein of interest, e.g. the mRNA may have sequences particularly susceptible to host endonucleases, which drastically reduce the functional lifetime of the mRNA, or the mRNA may form secondary structures that mask the start codon or ribosome binding site, thereby inhibiting initiation of translation in some hosts, (3) the selection, availability, and arrangement of host-compatible expression-control sequences in the 3'- and 5'-regions flanking the coding region -- these include promoters, 5'- and 3'-protector sequences, ribosome binding sites, transcription terminators, enhancers, polyadenylate addition sites, cap sites, intron-splice sites, and the like, (4) whether the protein has a secretion-signal sequence which can be processed by the host, or whether an expression-control sequence encoding a signal sequence endogenous to the host must be spliced onto the region encoding the mature protein, (5) the available modes and efficiencies of transfection or transformation of the host, and whether transient or stable expression is desired, (6) the scale and cost of the host culture system desired for expressing the protein, (7) whether, and what type of, posttranslational modifications are desired, e.g. the extent and kind of glycosylation desired may affect the choice of host, (8) the ease with which the expressed protein can be separated from proteins and other materials of the host cells and/or culture medium e.g. in some cases it may be desirable to express a fusion protein with a specialized signal sequence to aid in later purification steps, e.g. Sassenfeld et al., Biotechnology, January 1984, (9) the stability and copy number of a particular vector in a selected host, e.g. Hofschneider et al., eds. Gene Cloning in Organisms Other than E. coli (Springer Verlag, Berlin, 1982), and (10) like factors known to those skilled in the art.

Many reviews are available which provide guidance for making choices and/or modifications of specific expression systems in light of the recited factors, e.g., de Boer and Shepard, "Strategies for Optimizing Foreign Gene Expression in Escherichia coli" pgs. 205-247, in Kroon, ed. Genes: Structure and Expression (John Wiley & Sons, New York, 1983), review several E. coli expression systems; Kucherlapati et al., Critical Reviews in Biochemistry, Vol. 16, Issue 4, pgs. 349-379 (1984), and Banerji et al., Genetic Engineering, Vol. 5, pgs. 19-31 (1983) review methods for transfecting and transforming mammalian cells; Reznikoff and Gold, eds., Maximizing Gene Expression (Butterworths, Boston, 1986) review selected topics in gene expression in E. coli, yeast, and mammalian cells; and Thilly, Mammalian Cell Technology (Butterworths, Boston, 1986) reviews mammalian expression systems.

Likewise, many reviews are available which describe techniques and conditions for linking and/or manipulating specific cDNAs and expression control sequences to create and/or modify expression vectors suitable for use with the present invention: e.g. Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, N.Y., 1982); Glover, DNA Cloning: A Practical Approach, Vol. I and II (IRL Press, Oxford, 1985), and Perbal, A Practical Guide to Molecular Cloning (John Wiley & Sons, N.Y., 1984).

Suitable expression systems for the invention include those disclosed by Itakura and Riggs, U.S. patent 4,704,362 (bacterial expression), by Clark et al., U.S. patent 4,675,285 and by Hamer U.S. patent 4,599,308 (mammalian expression), and by Kurjan et al., U.S. patent 4,546,082 (yeast expression). Accord-

ingly, the above patents are incorporated by reference.

Whenever SV40-based vectors are used, e.g. pcD vectors, a preferred host is the COS7 cell line, described by Gluzman, Cell, Vol. 23, pgs. 175-182 (1981) and available from the ATCC under accession number CRL1651.

Soluble FcγRIIIs of the invention are administered as a pharmaceutical composition for treating ITP. Such compositions contain an effective amount of the FcγRIII in a pharmaceutical carrier. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient. Generally, compositions useful for parenteral administration of such drugs are well known, e.g. Remington's Pharmaceutical Science, 15th Ed. (Mack Publishing Company, Easton, PA 1980). Alternatively, compositions of the invention may be introduced into a patient's body by an implantable drug delivery system, e.g. Urquhart et al., Ann. Rev. Pharmacol. Toxicol., Vol. 24, pgs. 199-236 (1984).

When administered parenterally, the soluble FcγRIIIs will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutical carrier. Such carriers are inherently non-toxic and non-therapeutic. Examples of such carriers are normal saline, Ringer's solution, dextrose solution, and Hank's solution. Nonaqueous carriers such as fixed oils and ethyl oleate may also be used. A preferred carrier is 5% dextrose/saline. The carrier may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, e.g., buffers and preservatives. The soluble FcγRIII is preferably formulated in purified form substantially free of aggregates and other proteins at a concentration in the range of about 5 to 30 mg/ml, and preferably at a concentration in the range of about 10 to 20 mg/ml.

Selecting an administration regimen to deliver to a patient an amount of soluble FcγRIII which is effective in ameliorating the thrombopenia associated with ITP depends on several factors, including the serum turnover rate of the soluble FcγRIII, the serum level of competing endogenous FcγRIII associated with the immune disorder, the possible immunogenicity of the soluble FcγRIII, and the like. Preferably, an administration regimen maximizes the amount of soluble FcγRIII delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of soluble FcγRIII delivered depends in part on the particular soluble FcγRIII employed and the severity of the disease being treated. Guidance in selecting appropriate doses is found in the literature on therapeutic uses of antibodies and antibody fragments, which are polypeptides of roughly the same size as the soluble FcγRIIIs, e.g. Bach et al., chapter 22, in Ferrone et al., eds., Handbook of Monoclonal Antibodies (Noges Publications, Park Ridge, NJ, 1985); and Russell, pgs. 303-357, and Smith et al., pgs. 365-389, in Haber et al., eds. Antibodies in Human Diagnosis and Therapy (Raven Press, New York, 1977). Preferably, the dose is in the range of about 1-20 mg/kg per day, more preferably about 1-10 mg/kg per day.

EXAMPLES

The following examples serve to illustrate the present invention. Selection of vectors and hosts as well as the concentration of reagents, temperatures, and the values of other variables are only to exemplify application of the present invention and are not to be considered limitations thereof.

Example I. Construction of stable mammalian cell transformants which express human FcγRIII

Preferably, mammalian cell lines capable of stable expression of the FcγRIII are produced by cotransfecting a host mammalian cell with a vector carrying a selectable marker and a vector carrying a host-compatible promoter and the FcγRIII cDNA insert. For pcD(SRα)-GP5, suitable hosts include Chinese hamster ovary cells, COS monkey cells, and mouse L cells, such as a thymidine kinase deficient mutant (tk^-) L cell available from the American Type Culture Collection under accession number CCL 1.3. The selectable marker allows one to select host cells which have a high probability of containing the FcγRIII gene fully integrated into the host genome. Typically, the ratio of pcD(SRα)-GP5 to the marker-containing vector in the transfection solution is about 10:1. Thus, if the marker gene is integrated into the host genome, it is very likely that pcD(SRα)-GP5 will also be integrated by virtue of its higher concentration. The selectable marker also provides a means of preventing the cultures of desired transformants from being overgrown by revertant cells.

tk^- mouse L cells are cotransfected with pcD(SRα)-GP5 and pSV2tk, a pSV2 plasmid carrying a thymidine kinase gene under control of the SV40 early promoter. The pSV2 plasmid is described by Mulligan et al., Science, Vol. 209, pgs. 1422-1427 (1980), and by Subramani et al., Mol. Cell. Biol., Vol. 1, pgs. 854-864 (1981), and is available from the American Type Culture Collection under accession number 37146. Both plasmids are amplified in E. coli, e.g. strain HB101 available from the ATCC under accession number 33694, and purified by cesium chloride equilibrium centrifugation. A suspension of abut 1 x 10^5 of tk^- L cells in 10 ml of Dulbecco's Modified Eagle Medium (DME) with 10% fetal bovine serum is placed in a Falcon 3003 dish and cultured at 37°C for 20 hours in a 5% carbon dioxide gas incubator, after which the medium is replaced by 10 ml of fresh DME with 10% fetal bovine serum. The culture is incubated for an additional 4 hours. After incubation, 0.5 ml of solution A (50 mM Hepes, 280 mM NaCl, 1.5 mM sodium phosphate buffer, pH 7.22) and 0.5 ml of solution B (2M $CaCl_2$, 10 μg pcD(SRα)-GP5, 1 μg pSV2tk) are added to the culture medium, and the culture is incubated at 37°C for 24 hours in a 5% CO_2 atmosphere, after which the cells are placed in a selective medium with HAT (e.g. Sigma Chemical Co., St. Louis,

MO). After two weeks the surviving colonies are subcloned by limiting dilution, and clones are assayed for expression of $Fc\gamma RIII$.

Example II. Use of stable L cell transformant expressing membrane-bound $Fc\gamma RIII$ to measure serum levels of immune complex

A stably transformed mammalian cell expressing the $Fc\gamma RIII$ of the invention can replace the Raji cell line in assays for immune complexes, e.g. Theofilopoulos et al., chapter 28, Manual of Clinical Laboratory Immunology, 3rd Ed. (American Society for Microbiology, Washington, D.C. 1986).

Antiserum to human IgG is prepared in rabbits, and the IgG fraction is isolated by ammonium sulfate precipitation followed by fractionation on an anion-exchange chromatography column (DEAE-cellulose 52; Whatman Chemical Separation Ltd., Maidstone, England). Antiserum from commercial sources may also be used. The IgG fraction of the antiserum is brought to 5 mg/ml, and 1 ml is labeled with ^{125}I. After jodination and dialysis, the antiserum is diluted to 1 mg/ml with phosphate-buffered saline (PBS) to give a specific activity of about 3 x 10^5 cpm/µg. Cells transformed with the $Fc\gamma RIII$ cDNA are harvested after 72 hours of culture, and portions of 2 x 10^6 cells in 200 µl of medium are placed in 1.5-ml plastic Eppendorf conical tubes (Brinkmann Instruments, Inc., Westbury, N.Y.; catalog no. 22-36-411-1). One ml. of Spinner medium (Eagle minimal medium without Ca^{2+} and Mg^{2+}) is added to each tube, and the cells are centrifuged at 800 x g for 8 min. Supernatant fluids are aspirated, and the cell pellets are resuspended in 50 µl of Spinner medium. Serum to be tested is diluted fourfold in 0.15 M NaCl (physiological saline), and 25 µl is added to the transformed cells. After a 45-min incubation period at 37°C with gentle shaking by hand every 5 to 10 min, the cells are washed three times with Spinner medium. After the final wash, the cells, gently shaken every 5 to 10 minutes, are allowed to react for 30 min at 4°C with ^{125}I-labeled rabbit anti-human IgG diluted with Spinner medium containing 10 g of human serum albumin (HSA) per liter. After incubation, the cells are washed three times, supernatant fluids are completely aspirated, and radioactivity of the cell pellet is determined in a gamma counter. All assays are done in duplicate. The amount of uptake, expressed as absolute counts, percentage of the input, or micrograms of antibody, is referred to a standard curve of radioactive antibody uptake by cells incubated with normal human serum (complement source) containing various amounts of aggregated human IgG (AHG). The quantity of immune complex in serum is equated to an amount of AHG after correction for the dilution factor and is expressed as micrograms of AHG equivalent per milliliter of serum. The soluble AHG is formed from a solution of 6.5 mg of Cohn fraction II or isolated human IgG per ml of physiological saline, heated at 63°C for 30 min, and centrifuged (1,500 x g, 15 min) to remove insoluble large aggregates. The supernatant is then diluted with buffer to yield a final concentration of approximately 1.6 mg/ml. Portions (0.5 ml) of this preparation are stored at -70°C and can be used for as long as 1 month.

The standard curve of radioactive antibody uptake is constructed as follows. Fifty-microliter portions of AHG (80 µg of protein) are serially diluted (11 twofold dilutions) in saline. Subsequently, 50 µl of a twofold dilution of normal human serum (source of complement), freshly obtained or stored at -70°C, is added to each dilution of AHG, mixed carefully, and incubated at 37°C for 30 min. Thereafter, 25 µl of each mixture is added to 2 x 10^5 cells in duplicate (fourfold final dilutions of serum containing from 20 µg to about 20 ng of AHG); the mixture is incubated, washed, and reacted with radiolabeled antibody. Radioactivity is then counted as with the test sera. A base line of radioactive antibody uptake (background) by cells incubated with 25 µl of a fourfold dilution of normal human serum, used as a source of complement in the reference curve, is also established.

Standard preparatins of IgG aggregates and of tetanus toxoid-human anti-tetanus toxoid immune complexes have recently been developed under the auspices of the International Union of Immunologists and are available through the Swiss Red Cross Blood Transfusion Service, e.g. Nydegger et al., Clin. Exp. Immunol., Vol. 58, pgs. 502-509 (1984).

Example III. Construction of a soluble human $Fc\gamma RIII$

Soluble $Fc\gamma RIII$ was constructed using site-specific oligonucleotide-directed mutagenesis of the $Fc\gamma RIII$ cDNA insert of pcD(SRα)-GP5. The entire cDNA insert of pcD(SRα)-GP5 was subcloned as a 2.4 kilobase Bam HI fragment into the Bluescript KS plasmid (Stratagene, San Diego, CA) and single-stranded DNA was then prepared.

A synthetic oligonucleotide (minimum size about 18 nucleotides) encoding a TAA stop codon and a Bam HI site (with mutations shown in boldface in Figure 2) was used as a primer for complementary strand synthesis using DNA polymerase I large fragment (Amersham, Arlington Heights, IL). Figure 2 indicates the position of the stop codon and Bam HI site relative to the major domains encoded by the $Fc\gamma RIII$ cDNA: S represents the region encoding the signal peptide, EC represents the region encoding the extracellular domain of $Fc\gamma RIII$, H represents the region encoding the hydrophobic, or transmembrane, domain of $Fc\gamma RIII$, and C represents the region encoding the cytoplasmic domain. Introducing the stop codon as indicated by Figure 2 produces a mutant $Fc\gamma RIII$ lacking both the cytoplasmic and transmembrane domains; it is soluble as shown by the designation "s$Fc\gamma RIII$" beside the modified DNA sequence. After the identity of the mutant was confirmed by DNA sequence analysis, it was subcloned back into the Bam HI site of the pcD(SRα) vector, amplified in E. coli, and transfected into COS 7 cells by electroporation. One day prior to transfection, approximately (1.5-2.0) x 10^6

COS 7 monkey cells were seeded onto individual 100 mm plates in Dulbecco's modified Eagle's medium (DME) containing 6% fetal calf serum and 2 mM glutamine. To perform the transfection, cells were harvested by trypsinization and counted, washed twice in serum-free DME, and suspended to (1-7) x 10^6 cells per ml in serum-free DME. DNA was added to 25 µg/ml and the mixture was allowed to stand at room temperature for 10 minutes, after which 0.8 ml was pulsed in a 0.4 cm sterile cuvette with a Bio Rad (Richmond, CA) Gene Pulser at 250 volts and 960 microfarads. After pulsing, cells were allowed to stand for 10 minutes and then were plated at (1.5-2.0) x 10^6 per 100 mm plate in DME plus 6% fetal calf serum. Supernatants were harvested and assayed for soluble FcγRIII after 72 hours.

The soluble FcγRIII in the COS7 culture supernatants was analyzed further by gel electrophoresis, and immunoprecipitated. The results of this are illustrated in Figure 3. Lanes 1, 3 and 6 each contain proteins from culture supernatants of COS7 cells transfected with the pcD carrying the soluble FcγRIII cDNA. Lane 1 proteins were immunoprecipitated with a non-specific mouse IgG_2 antibody; Lane 3 proteins were immunoprecipitated with human IgG, presumably by the binding of the Fc portion of the antibody with the soluble FcγRIII; and Lane 6 proteins were immunoprecipitated with the monoclonal antibody 3G8 which is specific for the extracellular domain of FcγRIII. Size markers adjacent to the Control lane (lane 1) are in kilodaltons. The soluble FcγRIII corresponds to the broad band at about 40 kilodaltons. Lanes 4 and 7 contain proteins from culture supernatants of COS7 cells transfected with the pcD carrying the soluble FcγRIII cDNA and cultured in the presence of tunicamycin. Tunicamycin inhibits the post-translational attachment of N-linked carbohydrates to proteins. It was applied in an attempt to determine the nature of the apparent heterogeneity of the 40 kilodalton band. As shown in Figure 2, the tunicamycin causes the appearance of two bands of lower molecular weight, which is consistent with the deglycosylation of the soluble FcγRIII. Tunicamycin was added to the cultures as disclosed by Martens et al., PNAS 84: 809-813 (1987). Lanes 2 and 5 contain proteins from culture supernatants of COS7 cells which had undergone the same manipulations as the COS cells of lanes 3 and 4 and lanes 6 and 7, respectively, with the exception that no plasmid DNA was included in the transfection protocol.

Example IV Isolation of a Variant human FcγRIII from Natural Killer Cells.

A cDNA library was constructed from mRNA extracted from a human natural killer (NK) cell line using the pcD(SRα) expression vector. The library was screened with a probe constructed from the cDNA insert of pcD(SRα)-GP5, which was radiolabeled with ^{31}P by random-primed DNA labeling (Boehringer Mannheim, Indianapolis, IN). A clone pcD(SRα)-NL10 was obtained that exhibited human IgG binding activity. The sequence of the cDNA insert of pcD(SRα)-NL10 is illustrated in Figure 4. It can be seen that the amino acid sequence of this FcγRIII and that encoded by pcD(SRα)-GP5 are very similar, differing in the extracellular domain by only six amino acids.

The descriptions of the foregoing embodiments of the invention have been presented for purpose of illustration and description. They are not intended to be exhaustive or to limit the invention to the precise forms disclosed, and obviously many modifications and variations are possible in light of the above teaching. The embodiments are chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with such modifications as are suited to the particular use contemplated. It is intended that the scope of the invention be defined by the claims appended hereto.

Applicants have deposited pcD(SRα)-GP5 with the American Type Culture Collection Rockville, MD, USA (ATCC), under accession number 67707. This deposit is made under the Budapest Treaty for the Deposit of Microorganisms; and also under conditions as provided under ATCC's agreement for Culture Deposit for Patent Purposes, which assures that the deposit will be made available to the US Commissioner of Patents and Trademarks pursuant to 35 U.S.C. 122 and 37 C.F.R. 1.14, and will be made available to the public upon issue of a U.S. patent, which requires that the deposit be maintained. Availability of the deposited strain is not to be contrued as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Claims

1. Soluble or membrane-bound human FcγRIII.

2. FcγRIII according to claim 1 having the amino acid sequence of Formula I or II.

3. A variant of FcγRIII according to claim 2, wherein the FcγRIII has the sequence of Formula I or II with 0 or 1 amino acid residue deleted and 0, 1 or 2 amino acid residues substituted according to the following table, and wherein there is at least one deletion or at least 1 substitution:

amino acid in Formula I or II	substituted by
Ala	Ser, Thr or Gly
Arg	Lys
Asn	Asp or Ser
Asp	Glu or Asn
Gln	Glu
Glu	Asp or Gln
Gly	Ala or Ser
His	Asn
Ile	Val or Leu
Leu	Val or Ile
Lys	Arg or Asn
Met	Leu
Pro	Ala
Ser	Ala, Thr, Gly or Asn
Thr	Ser, Ala or Lys
Val	Ile, Ala or Leu.

4. A variant of $Fc\gamma RIII$ according to claim 2, wherein the $Fc\gamma RIII$ has the sequence of Formula I or II with 1 or 2 amino acid residues substituted according to the following table:

amino acid in Formula I or II	substituted by
Ala	Ser
Asn	Asp
Asp	Glu
Glu	Asp
Gly	Ala
Ile	Val
Leu	Val
Lys	Arg
Ser	Ala or Thr
Thr	Ser or Ala
Val	Ile.

5. A protein consisting of the extracellular domain of a human $Fc\gamma RIII$.

6. A pharmaceutical composition comprising an effective amount of $Fc\gamma RIII$ according to any of Claims 1-4 or a protein according to Claim 5, and a pharmaceutically acceptable carrier.

7. A pharmaceutical composition according to Claim 6 for treatment of immune thrombocytopenic purpura.

8. A pharmaceutical composition according to Claim 6 or Claim 7 comprising 5-30 mg/ml $Fc\gamma RIII$.

9. A pharmaceutical composition according to Claim 6 or Claim 7 comprising 10-20 mg/ml $Fc\gamma RIII$.

10. Use of $Fc\gamma RIII$ in manufacture of a medicament for treatment of thrombocytopenia.

11. Use of $Fc\gamma RIII$ in manufacture of a medicament for treatment of immune thrombocytopenic purpura.

12. Use according to Claim 10 or Claim 11 of $Fc\gamma RIII$ according to any of Claims 1-4 or of a protein according to Claim 5.

```
        10                  30                  50
CACTCCAGTGTGGCATCATGTGGCAGCTGCTCCTCCCAACTGCTCTGCTACTT
                  MetTrpGlnLeuLeuLeuProThrAlaLeuLeuLeu
       110                 130                 150
GTTCCTGGAGCCTCAATGGTACAGGGTGCTCGAGAAGGACAGTGTGACTCTG
lPheLeuGluProGlnTrpTyrArgValLeuGluLysAspSerValThrLeu
       210                 230                 250
CACAATGAGAACCTCATCTCAAGCCAGGCCTCGAGCTACTTCATTGACGCTGCC
HisAsnGluAsnLeuIleSerSerGlnAlaSerSerTyrPheIleAspAlaAla
       310                 330                 350
CCCTCAGTGACCCGGTGCAGCTAGAAGTCCATGTCGGCTGGCTGTTGCTCCAG
hrLeuSerAspProValGlnLeuGluValHisValGlyTrpLeuLeuLeuGln
       410                 430                 450
TCACAGCTGGAAGAACACTGCTCTGCATAAGGTCACATATTTACAGAATGGC
sHisSerTrpLysAsnThrAlaLeuHisLysValThrTyrLeuGlnAsnGly
       510                 530                 550
GCCACACTCAAAGATAGCGGCTCCTACTTCTGCAGGGGGCTTGTTGGGAGTAAA
AlaThrLeuLysAspSerGlySerTyrPheCysArgGlyLeuValGlySerLys
       610                 630                 650
CAGTGTCAACCATCTCATCATTCTCTCCACCTGGGTACCAAGTCTCTTTCTGC
laValSerThrIleSerSerPheSerProProGlyTyrGlnValSerPheCys
       710                 730                 750
TGTGAAGACAAACATTTGAAGCTCAACAAGAGACTGGAAGGACCATAAACTTA
rValLysThrAsnIleEnd

AATAAGAGCAGTGGCAGCAGCATCTCTGAACATTTCTCTGGATTTGCAACCCC
GCCAGATCCTTTATCCAACTCTCGATTTTTCCTTGGTCTCCAGTGGAAGGGAA
CTAGAAATTGAAGTTTCACAGCTACACAAACACTTTTTCTGTCCCAACCATTC
CATACAAAAATTGCTCGTATTATAAATTACCCAGTTTAGAGGGGAAAAAAGAA
AGGACCCTACAGAGTGTGGGAACTGCTGGGGATCTAGAGAATTCAGTGGGACC
GGGAGGTGTTCCCATCTGAGCCCAGAGATAAGGGTGTCTTCCTAGAACATTAG
CTTCCAGGGGACTCTATCAGAACTGGACCATTTCCAAGTATATAACGATGAGC
TTGGGGTGGGGGTGGGGTGGAAAAGAAAGTACAGAACAAACCCTGTGTCACTG
AATGAGAAAGCCTGAGAAGAAAGAACCAACCACAAGCACACAGGAAGGAAAGC
TTAATGCAGGGACTGTAAAACCACCTTTTCTGCTTCAATGTCTAGTTCCTGTA
TAAATGTACTACTGAGCTTCACTGAGTTACGCTGTGAAACTTTCAAATCCTTC
ATGAAAAAAAGCTTTAGCTGTCTCCTGTTTTGTAAGCTTTCAGTGCAACATTT
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FIGURE 1 PART A

```
                70  +1                90
 CTAGTTTCAGCTGGCATGCGGACTGAAGATCTCCCAAAGGCTGTGGT
 LeuValSerAlaGlyMetArgThrGluAspLeuProLysAlaValVa
                170                190
AAGTGCCAGGGAGCCTACTCCCCTGAGGACAATTCCACACAGTGGTTT
LysCysGlnGlyAlaTyrSerProGluAspAsnSerThrGlnTrpPhe
                270                290
  ACAGTCGACGACAGTGGAGAGTACAGGTGCCAGACAAACCTCTCCA
  ThrValAspAspSerGlyGluTyrArgCysGlnThrAsnLeuSerT
                370                390
 GCCCCTCGGTGGGTGTTCAAGGAGGAAGACCCTATTCACCTGAGGTG
 AlaProArgTrpValPheLysGluGluAspProIleHisLeuArgCy
                470                490
AAAGACAGGAAGTATTTTCATCATAATTCTGACTTCCACATTCCAAAA
LysAspArgLysTyrPheHisHisAsnSerAspPheHisIleProLys
                570                590
  AATGTGTCTTCAGAGACTGTGAACATCACCATCACTCAAGGTTTGG
  AsnValSerSerGluThrValAsnIleThrIleThrGlnGlyLeuA
                670                690
 TTGGTGATGGTACTCCTTTTTGCAGTGGACACAGGACTATATTTCTC
 LeuValMetValLeuLeuPheAlaValAspThrGlyLeuTyrPheSe
                770                790
 AATGGAGAAAGGACCCTCAAGACAAATGACCCCCATCCCATGGGAGT


 ATCATCCTCAGGCCTCTCTACAAGCAGCAGGAAACATAGAACTCAGA
 AAGCCCATGATCTTCAAGCAGGGAAGCCCCAGTGAGTAGCTGCATTC   1000
 CCTCACAGTAAAACAACAATACAGGCTAGGGATGGTAATCCTTTAAA
 AATAATTATTCCTAAACAAATGGATAAGTAGAATTAATGATTGAGGC   1200
 AATGAAAGCATGGCTGAGAAATAGCAGGGTAGTCCAGGATAGTCTAA
 CCGTAGTGGAATTAACAGGAAATCATGAGGGTGACGTAGAATTGAGT   1400
 CCTCTAATGCTAGGAGTAGCAAATGGTCCTAGGAAGGGGACTGAGGA
 TCCCAAGTTAAGCTAAGTGAACAGAACTATCTCAGCATCAGAATGAG   1600
 GCAGGAGGTGAAAATGCTTTCTTGGCCAGGGTAGTAAGAATTAGAGG
 TAGCTTTGTTCATTGCATTTATTAAACAAATGTTGTATAACCAATAC   1800
 TTCAGTCAGTTCCAATGAGGTGGGGATGGAGAAGACAATTGTTGCTT
 CTTGGTTCCAATAAAGCATTTTACA (A)n
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FIGURE 1 PART B

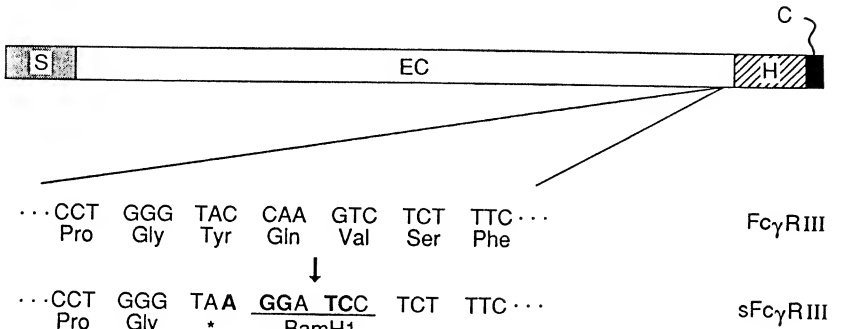
C
S
EC
H
···CCT GGG TAC CAA GTC TCT TTC···
Pro Gly Tyr Gln Val Ser Phe
FcγRIII
···CCT GGG TAA GGA TCC TCT TTC···
Pro Gly * BamH1
sFcγRIII

FIGURE 2

NL 10

```
       10                  30                  50
GGGGGGGGGGGGGGTAAATCCGCAGGACCTGGGTAACACGAGGAAGGGCTCCG

      110                 130                 150
TGCTCCTCCCAACTGCTCTGCTACTTCTAGTTTCAGCTGGCATGCGGACTGAA
euLeuLeuProThrAlaLeuLeuLeuValSerAlaGlyMetArgThrGlu
      210                 230                 250
GCTCGAGAAGGACAGTGTGACTCTGAAGTGCCAGGGAGCCTACTCCCCTGAG
lLeuGluLysAspSerValThrLeuLysCysGlnGlyAlaTyrSerProGlu
      310                 330                 350
GCCTCGAGCTACTTCATTGACGCTGCCACAGTCGACGACAGTGGAGACTACAGG
AlaSerSerTyrPheIleAspAlaAlaThrValAspAspSerGlyGluTyrArg
      410                 430                 450
TCCATATCGGCTGGCTGTTGCTCCAGGCCCCTCGGTGGGTGTTCAAGGAGGAA
alHisIleGlyTrpLeuLeuLeuGlnAlaProArgTrpValPheLysGluGlu
      510                 530                 550
TAAGGTCACATATTTACAGAATGGCAAAGGCAGGAAGTATTTTCATCATAAT
sLysValThrTyrLeuGlnAsnGlyLysGlyArgLysTyrPheHisHisAsn
      610                 630                 650
TTCTGCAGGGGGCTTTTTGGGAGTAAAAATGTGTCTTCAGAGACTGTGAACATC
PheCysArgGlyLeuPheGlySerLysAsnValSerSerGluThrValAsnIle
      710                 730                 750
CACCTGGGTACCAAGTCTCTTTCTGCTTGGTGATGCTACTCCTTTTTGCAGTG
roProGlyTyrGlnValSerPheCysLeuValMetValLeuLeuPheAlaVal
      810                 830                 850
AAGAGACTGGAAGGACCATAAATTTAAATGGAGAAAGGACCCTCAAGACAAATGA
rArgAspTrpLysAspHisLysPheLysTrpArgLysAspProGlnAspLysEnd

GAACATTTCTCTGGATTTGCAACCCCATCATCCTCAGGCCTCTCTACAAGCAGCA
TTTCCTTGGTCTCCAGTGGAAGGGAAAAGCCCATGATCTTCAAGCAGGGAAGCCC
AAACACTTTTTCTGTCCCAACCGTTCCCTCACAGCAAAGCAACAATACAGGCTAG
TACCCAGTTTAGAGGGGAAAAAAAAACAATTATTCCTAAATAAATGGATAAGTAG
GGGATCTAGGGAATTCAGTGGGACCAATGAAAGCATGGCTGAGAAATAGCAGGTA
AAGGGTGTCTTCCTAGAACATTAGCCGTAGTGGAATTAACAGGAAATCATGAGGG
ATCTCCAAGTATATAACGATGAGTCCTCTTAATGCTAGCAGTAGAAAATGGTCCT
AGTACAGAACAAACCCTGTGTCACTGTCCCAAGTTGCTAAGTGAACAGAACTATC
GCACACAGGAAGGGCGGCCAGGAGGTGAAAATGCTTTCTTGGCCAGGGTAGTAA
CAATATCTAATTCCTGTGTAGCTTTGTTCATTGCATTTATTAAACAAATGTTGTA
GAAACTTTCAAATCCTTCATCATGTCAGTTCCAATGAGGTGGGGATGGAGAAGAC
GCTTTAAGCGCAACATTTCTTGGTTCCAATAAAGCATTTTACAAGATCTTGCATG
ATGATAAAAAAAAAAAAAAAAAAAA
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FIGURE 3 PART A

NL 10

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                70                                  90
GATATCTTTGGTGACTTGTCCACTCCAGTGTGGCATCATGTGGCAGC
                                                  MetTrpGlnL
               170                                 190
GATCTCCCAAAGGCTGTGGTGTTCCTGGAGCCTCAATGGTACAGGGT
AspLeuProLysAlaValValPheLeuGluProGlnTrpTyrArgVa
               270                                 290
GACAATTCCACACAGTGGTTTCACAATGAGAGCCTCATCTCAAGCCAG
AspAsnSerThrGlnTrpPheHisAsnGluSerLeuIleSerSerGln
               370                                 390
  TGCCAGACAAACCTCTCCACCCTCAGTGACCCGGTGCAGCTAGAAG
  CysGlnThrAsnLeuSerThrLeuSerAspProValGlnLeuGluV
               470                                 490
GACCCTATTCACCTGAGGTGTCACAGCTGGAAGAACACTGCTCTGCA
AspProIleHisLeuArgCysHisSerTrpLysAsnThrAlaLeuHi
               570                                 590
TCTGACTTCTACATTCCAAAAGCCACACTCAAAGACAGCGGCTCCTAC
SerAspPheTyrIleProLysAlaThrLeuLysAspSerGlySerTyr
               670                                 690
  ACCATCACTCAAGGTTTGGCAGTGTCAACCATCTCATCATTCTTTC
  ThrIleThrGlnGlyLeuAlaValSerThrIleSerSerPhePheP
               770                                 790
GACACAGGACTATATTTCTCTGTGAAGACAAACATTCGAAGCTCAAC
AspThrGlyLeuTyrPheSerValLysThrAsnIleArgSerSerTh
               870                                 890
   CCCCCATCCCATGGGGGTAATAAGAGCAGTAGCAGCAGCATCTCT
```

```
   GGAAACATAGAACTCAGAGCCAGATCCCTTATCCAACTCTCGACT   1000
   CAGTGAGTAGCTGCATTCCTAGAAATTGAAGTTTCAGAGCTACAC
   GGATGGTAATCCTTTAAACATACAAAAATTGCTCGTGTTATAAAT   1200
   AATTAATGGTTGAGGCcAGGACCATACAGAGTGTGGGAACTGCTG
   GTCCAGGATAGTCTAAGGGAGGTGTTCCCATCTGAGCCCAGAGAT   1400
   TGACGTAGAATTGAGTCTTCCAGGGGACTCTATCAGAACTGGACC
   AGGAAGGGGACTGAGGATTGCGGTGGGGGGTGGGGTGGAAAAGAA   1600
   TCAGCATCAGAATGAGAAAGCCTGAGAAGAAAGAACCAACCACAA
   GAATTAGAGGTTAATGCAGGGACTGTAAAACCACCTTTTCTGCTT   1800
   TAACCAATACTAAATGTACTACTGAGCTTCGCTGAGTTAAGTTAT
   AATTGTTGCTTATGAAAGAAAGCTTTACGTGTCTCTGTTTTGTAA   2000
   CTACTCTTAGATAGAAGATGGGAAAACCATGGTAATAAAATATGA
```

FIGURE 3 PART B

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 97 10 6398

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	WO 88 06733 A (KHAYAT DAVID) 7 September 1988 * claims 1-5,20 *	1,2,5-12	C12N15/12 C07K14/735 A61K38/17
P,X	NATURE, JUN 9 1988, 333 (6173) P568-70, ENGLAND, XP002032511 SIMMONS D ET AL: "The Fc gamma receptor of natural killer cells is a phospholipid-linked membrane protein [published erratum appears in Nature 1989 Aug 24;340(6235):662]" * the whole document *	1,2,5-12	
A	J IMMUNOL METHODS, JUN 26 1987, 100 (1-2) P235-41, NETHERLANDS, XP002032512 KHAYAT D ET AL: "Soluble circulating Fc gamma receptors in human serum. A new ELISA assay for specific and quantitative detection." * the whole document *	1,2,5,12	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C12N
C07K

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
THE HAGUE	6 June 1997	Nauche, S

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EP 97106398 - C -

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.

Claims searched completely:	1,2,5,10-12
Claims searched incompletely:	6-9
Claims not searched:	3,4

Reason for the limitation of the search:

The amount of possible combinations of all variants disclosed in claims 3 and 4 is speculative : for either Formula, there are more than 3.10^{24} possible combinations in claim 3 and more than 2.10^{19} possible combinations in claim 4.Thesclaims do not meet the requirements for article 84 EPC.